

The role of RNA silencing in *Tospovirus* infection and transgenic resistance

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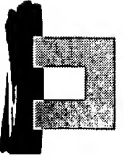
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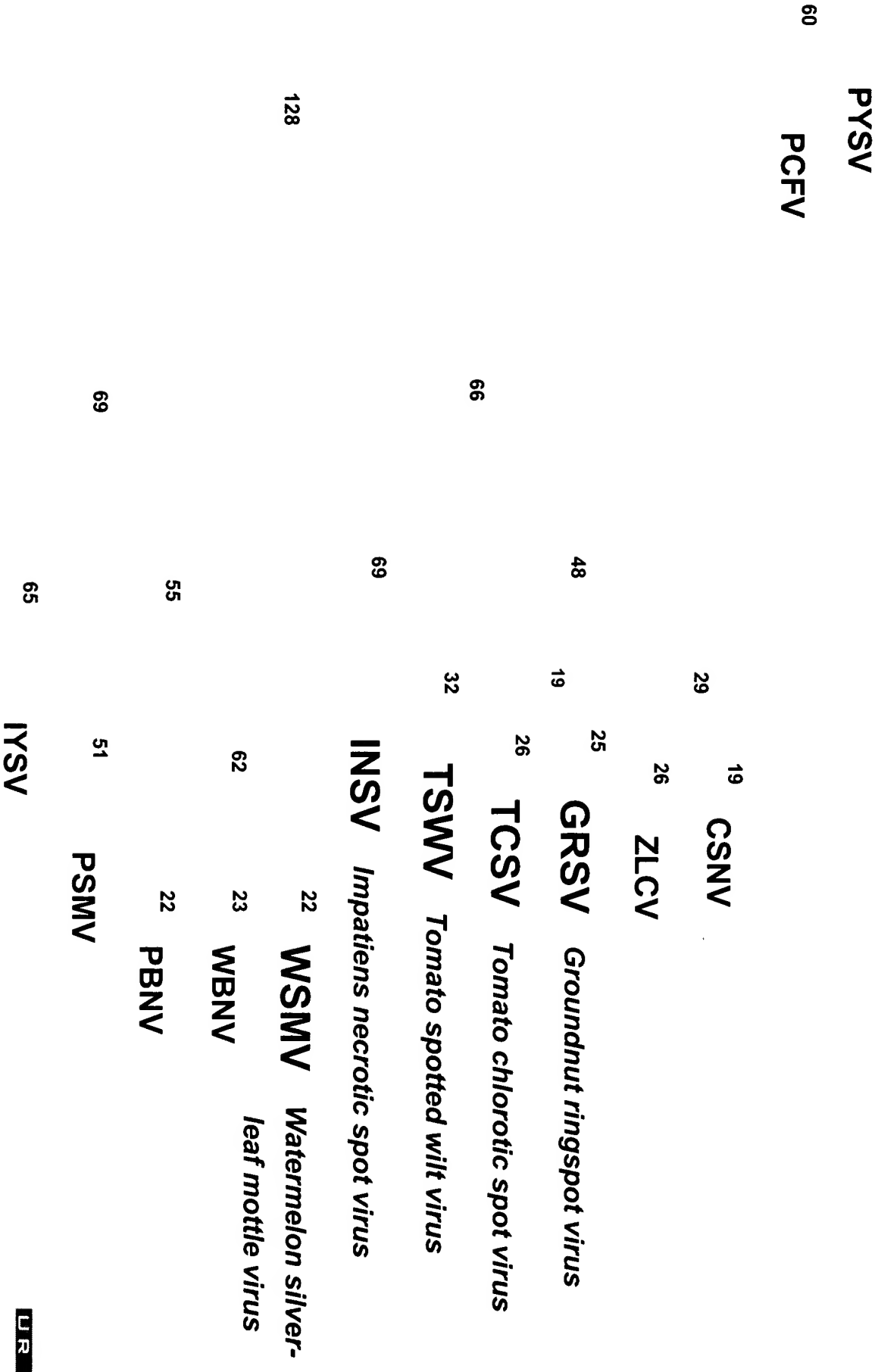
TOSPOVIRUSES (family *Bunyaviridae*)

Type species tomato spotted wilt virus (TSWV)

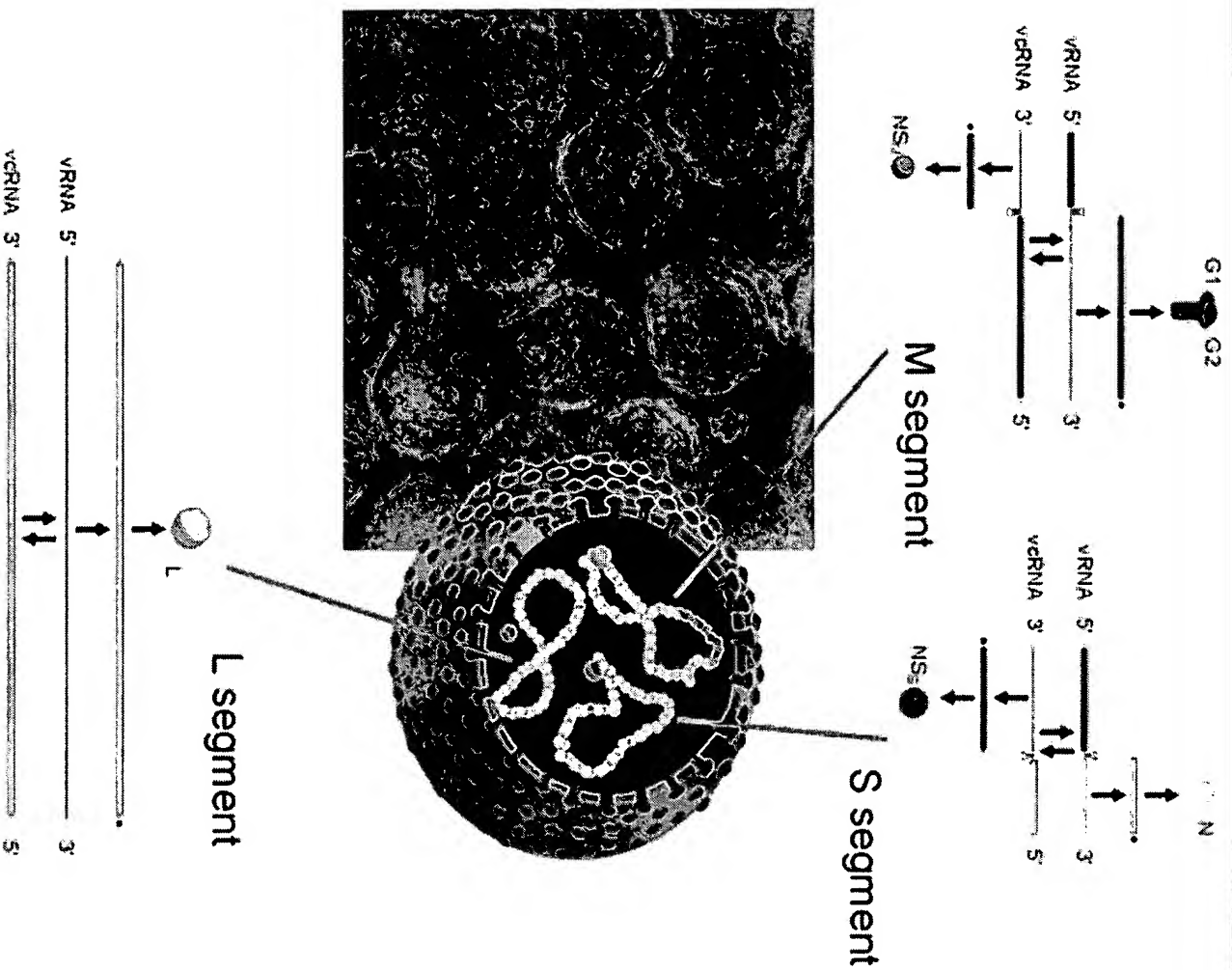
- ➔ **More than 10 established species**
- ➔ **Transmitted by thrips in a propagative manner**
- ➔ **Causal agents of major diseases in many economically important crops and ornamentals**



Tospovirus family



TSWV



The five genes of TSWV:

L polymerase

NS_M movement protein

G1/G2 glycoproteins

N nucleocapsid

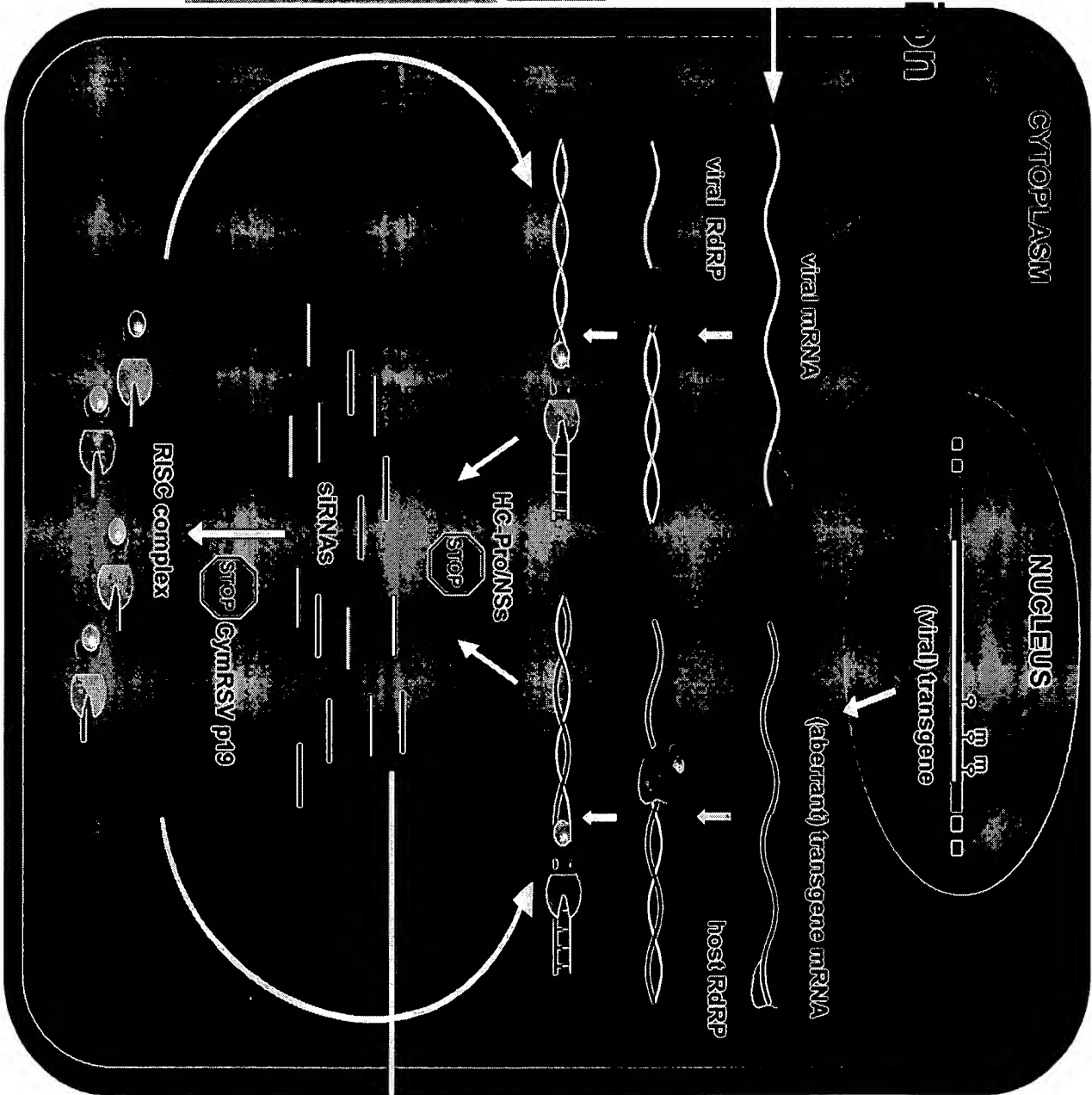
NS_s silencing suppressor



DTGS for virus resistance / silencing suppression

invading virus

aliana	C. elegans
assa	D. melanogaster
SGS3	
rp SDE1 SGS2 QDE1 EGO1	
lase Dicer/DCR Carpelfactory	
helicase SDE3	
Argonaute SGS4 AGO1 RDEQDE2	



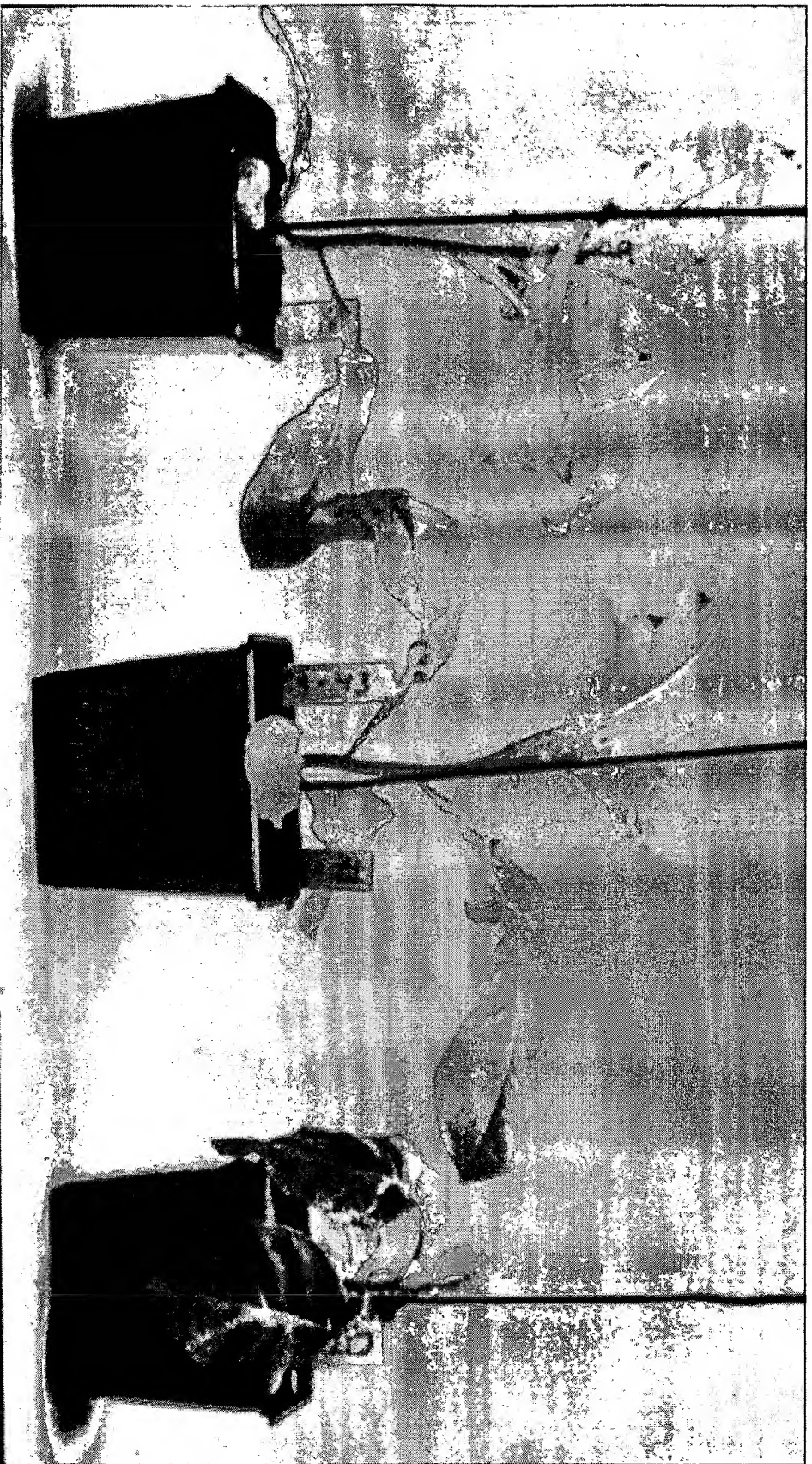
PVX p25
STOP

systemic sign:

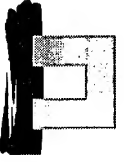


'The old days'

**RNA-mediated resistance; Plant Viruses, 1991
Post Transcriptional Gene Silencing (PTGS)**



(De Haan et al, 1991)



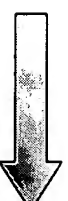
Post transcriptional gene silencing (PTGS)

- **Characterized by high transcription rates and a low steady-state RNA level**
 - **Posttranscriptional degradation in the cytoplasm**
- **Resistance is RNA-mediated (untranslatable mRNAs also confer resistance)**
- **Silencing is very sequence specific**
 - **Resistance is narrow**
- **dsRNA triggers silencing**



Transgenic resistance by PTGS

- Transgenic resistance can be obtained by the transformation of a (partial) viral gene into a plant
- Single gene silencing
 - <<10% of transformed plants
- Repeated sequences: inverted repeat
 - 60%
- Intron(-spliced) inverted repeats
 - 90%



Virus resistance against CMV

Constructs

Resistance in R_0



0% (11% in S_1)



75%

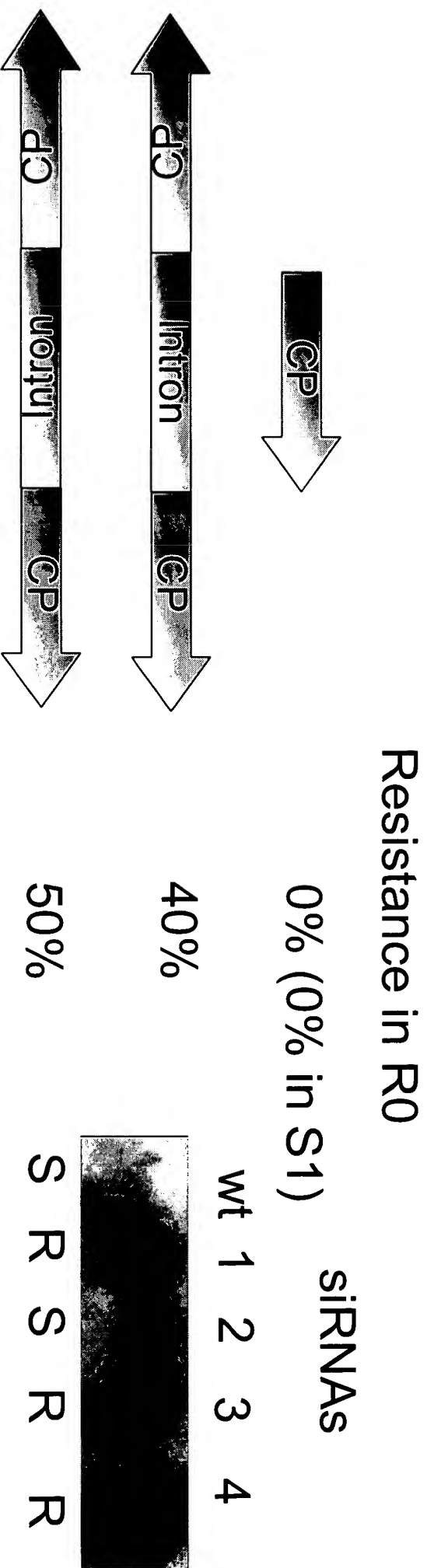
siRNAs



33%



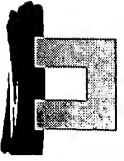
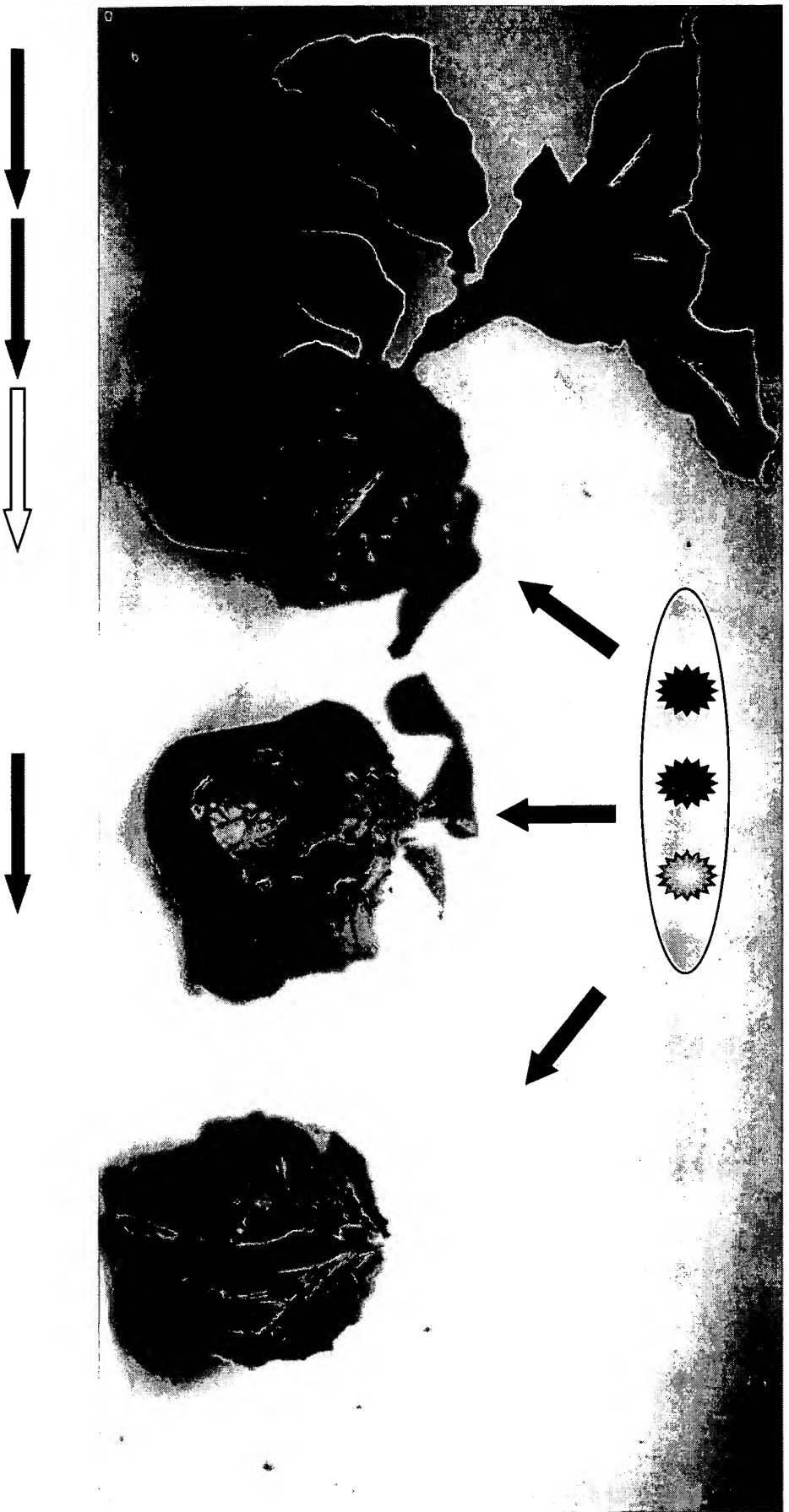
Virus resistance against CMV



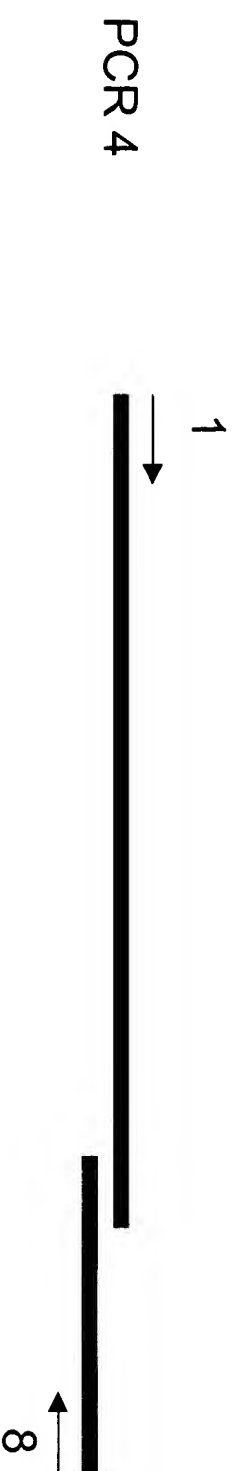
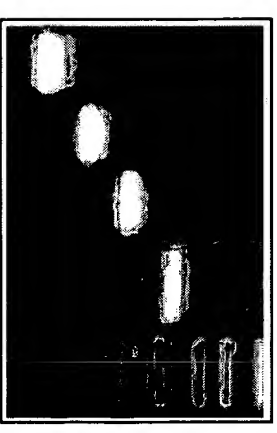
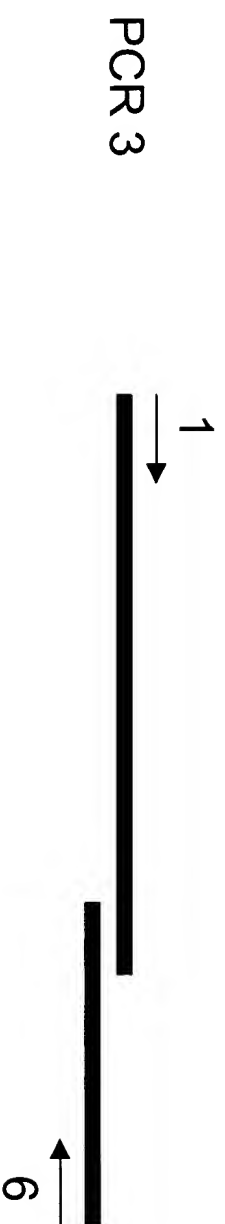
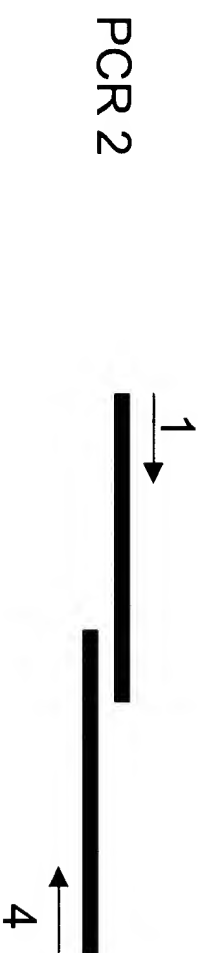
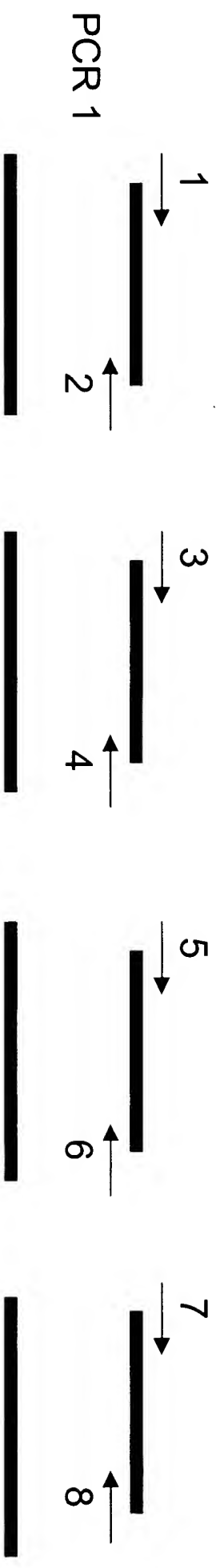
Resistance in the IR S1 lines was always 100%



Broad tospovirus resistance using multiple transgenes

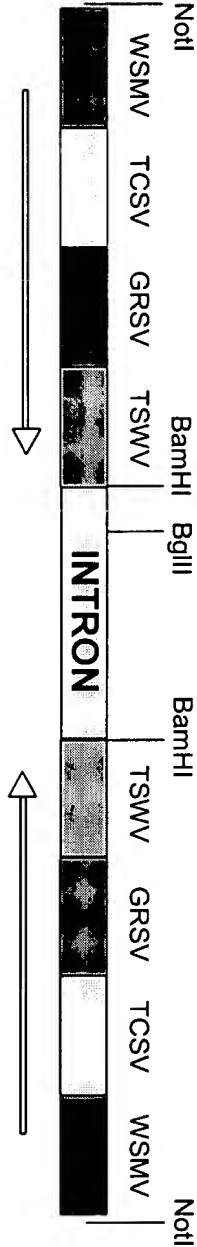


Using fusion PCR to create a multi-tospo construct

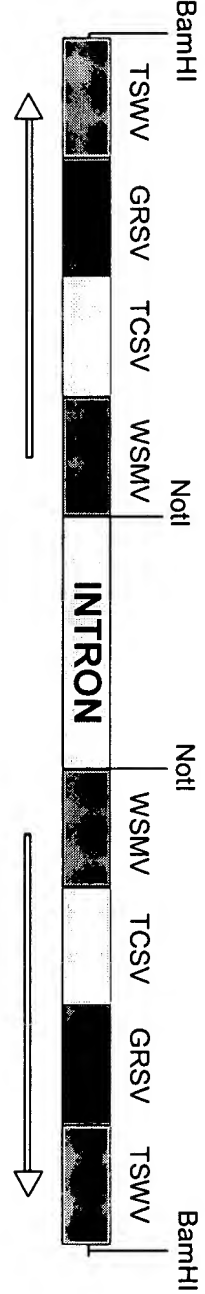


Tospoviral inverted repeat cassettes

IRN
SA

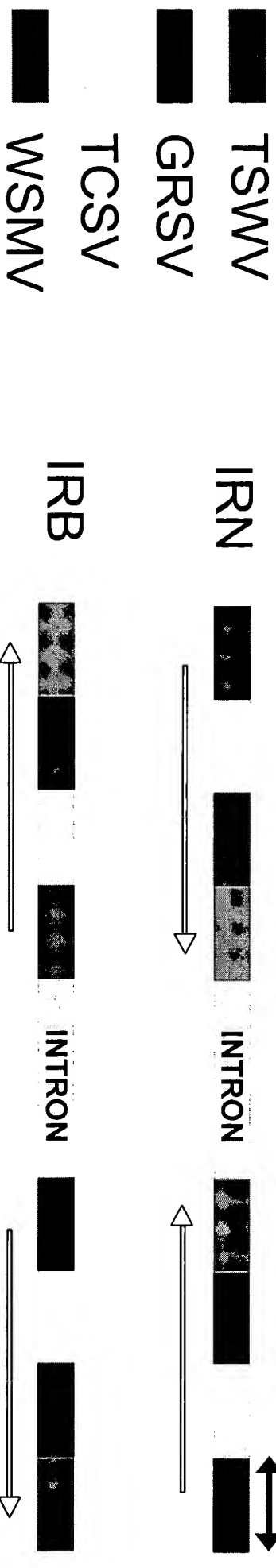


IRB
AS



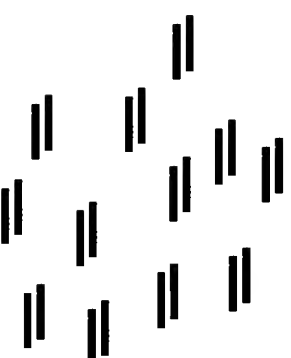
Tospoviral inverted repeat cassettes

N-gene segments:



dsRNA

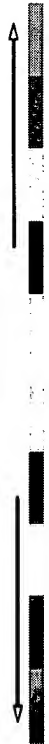
DICER



multiple virus resistance?



Resistance analysis of the IRB plants



Line name	TSWV	GRSV	TCSV	WSMV	Mix
IRB2	100%	N/A	N/A	N/A	N/A
IRB4	100%	100%	100%	100%	100%
IRB6	100%	100%	100%	100%	100%
IRB7	100%	100%	100%	100%	100%
IRB8	100%	100%	100%	100%	100%
IRB9	100%	100%	100%	100%	100%
IRB10	100%	100%	100%	100%	100%
IRB11	100%	100%	100%	100%	100%
IRB12	100%	100%	100%	100%	100%
IRB14	100%	100%	100%	100%	100%
IRB15	100%	100%	100%	100%	100%
IRB16	100%	100%	100%	100%	100%
IRB19	100%	100%	100%	100%	100%
IRB20	100%	100%	100%	100%	100%

82% of all IRB lines are resistant against all four tospoviruses



Resistance analysis: an example

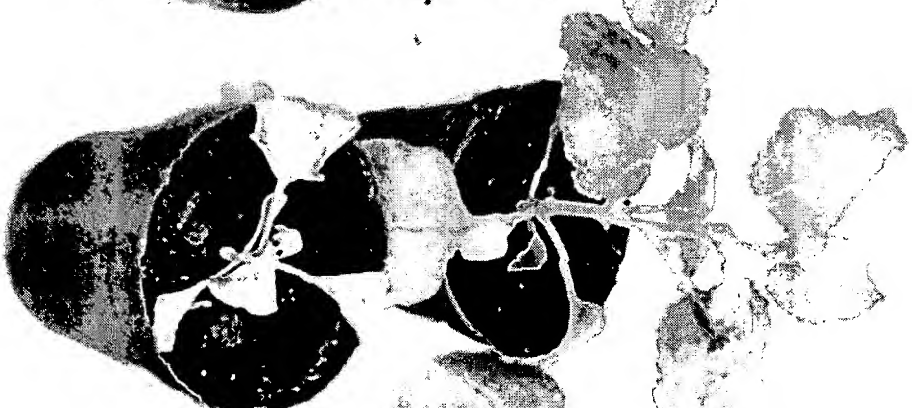
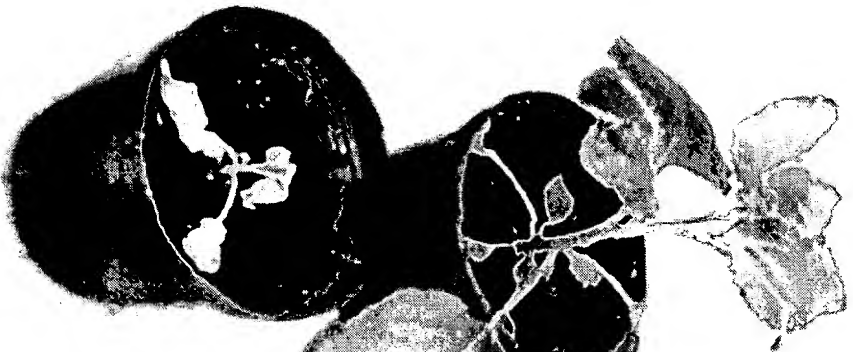
TSMV

GRSV

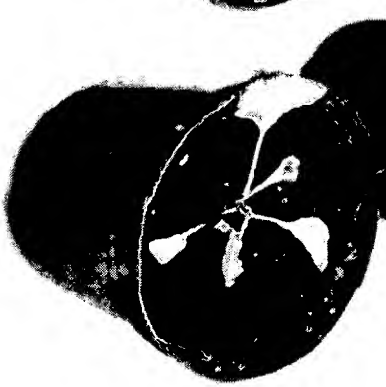
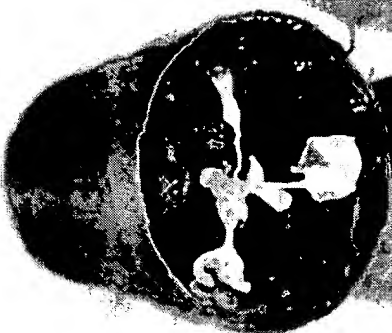
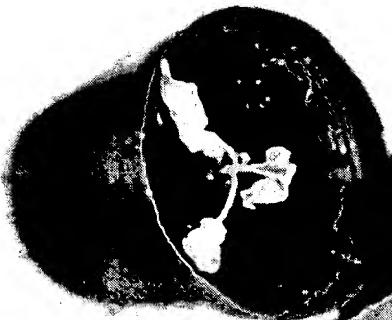
TCSV

WSMV

IRB16



IRB17



Resistance analysis of the IRN plants

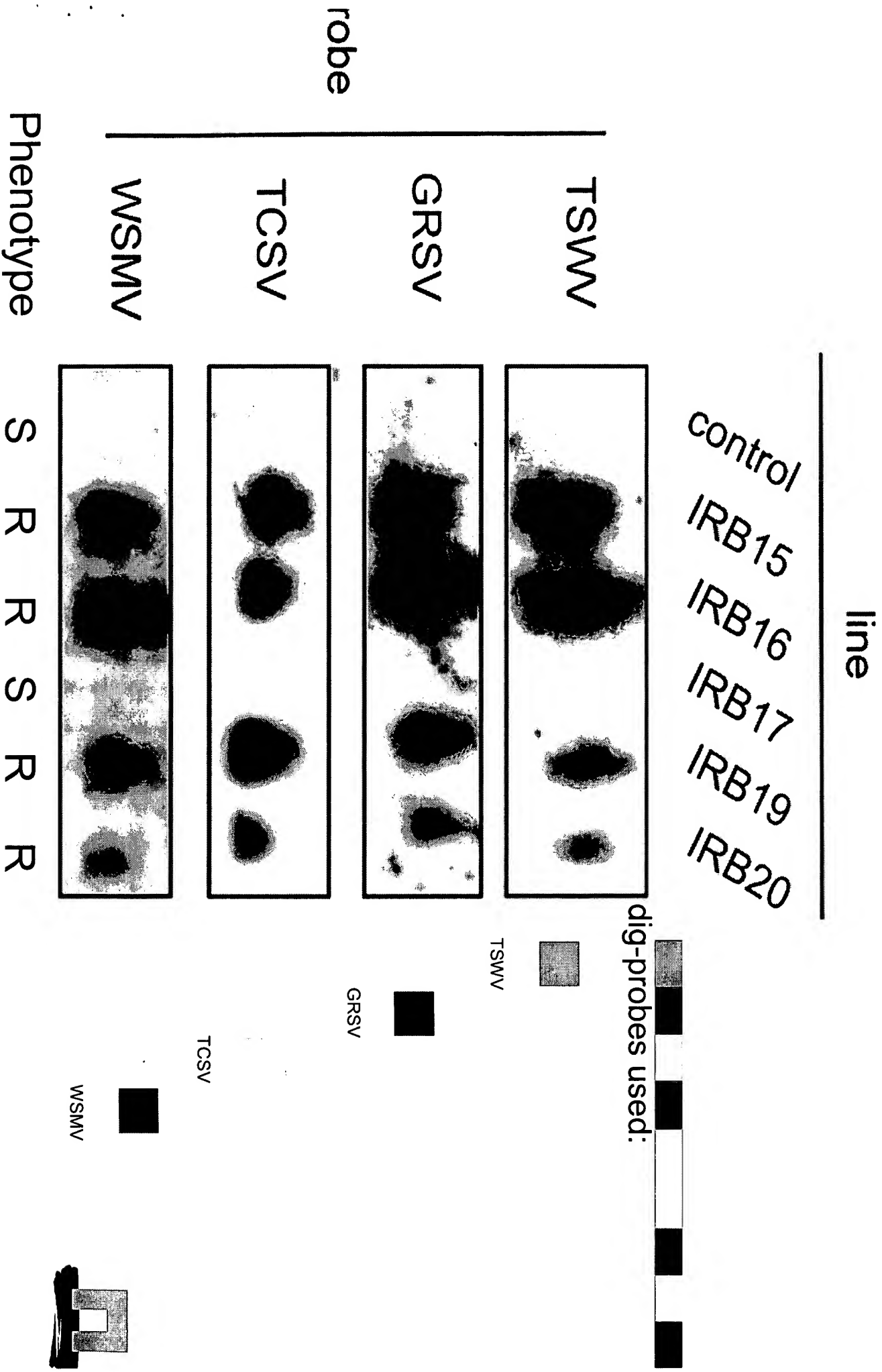


Line name	TSWV	GRSV	TCSV	WSMV	Mix
IRN6	66%	100%	100%	100%	100%
IRN8	100%	100%	100%	100%	100%
IRN9	100%	100%	100%	100%	100%
IRN10	100%	100%	100%	100%	100%
IRN14	66%	100%	100%	100%	100%
IRN15	100%	100%	100%	100%	100%
IRN19	100%	100%	100%	100%	66%
IRN20	100%	100%	100%	100%	66%
IRN21	0%	0%	0%	100%	0%

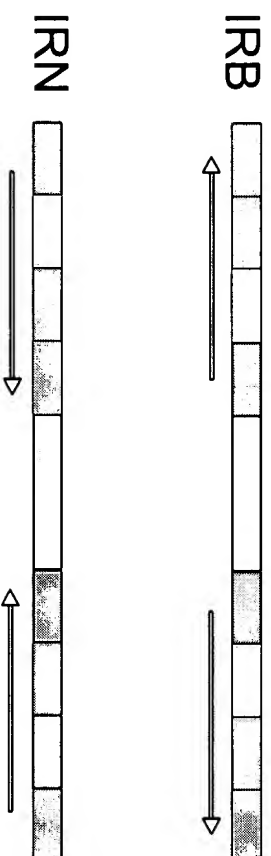
58% of all IRN lines were resistant against all four tospoviruses



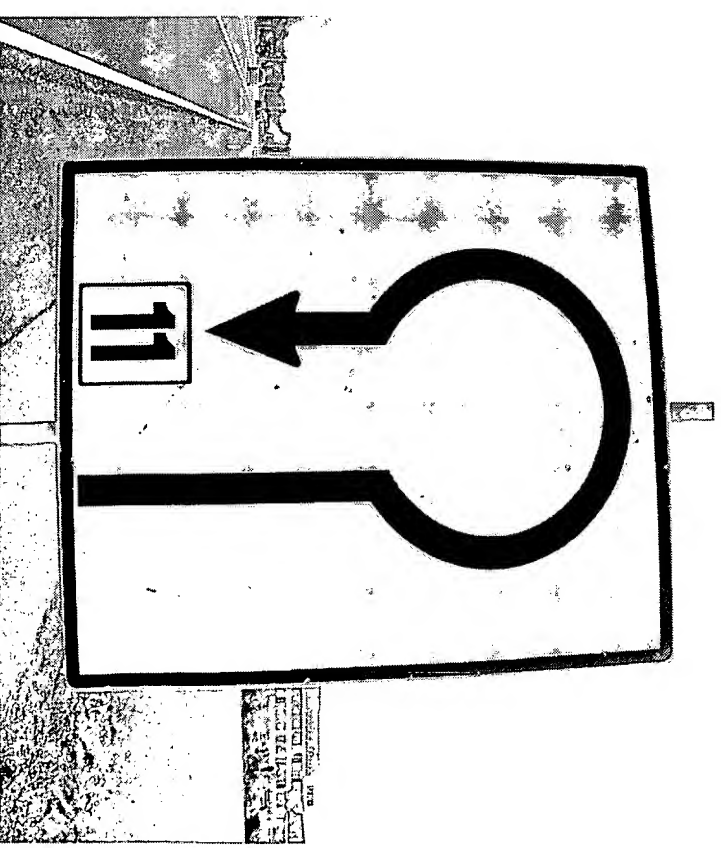
Molecular analysis of several IRB lines: transgenic 'viral' siRNAs



Resistance analysis



High levels of multiple virus resistance

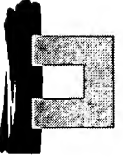
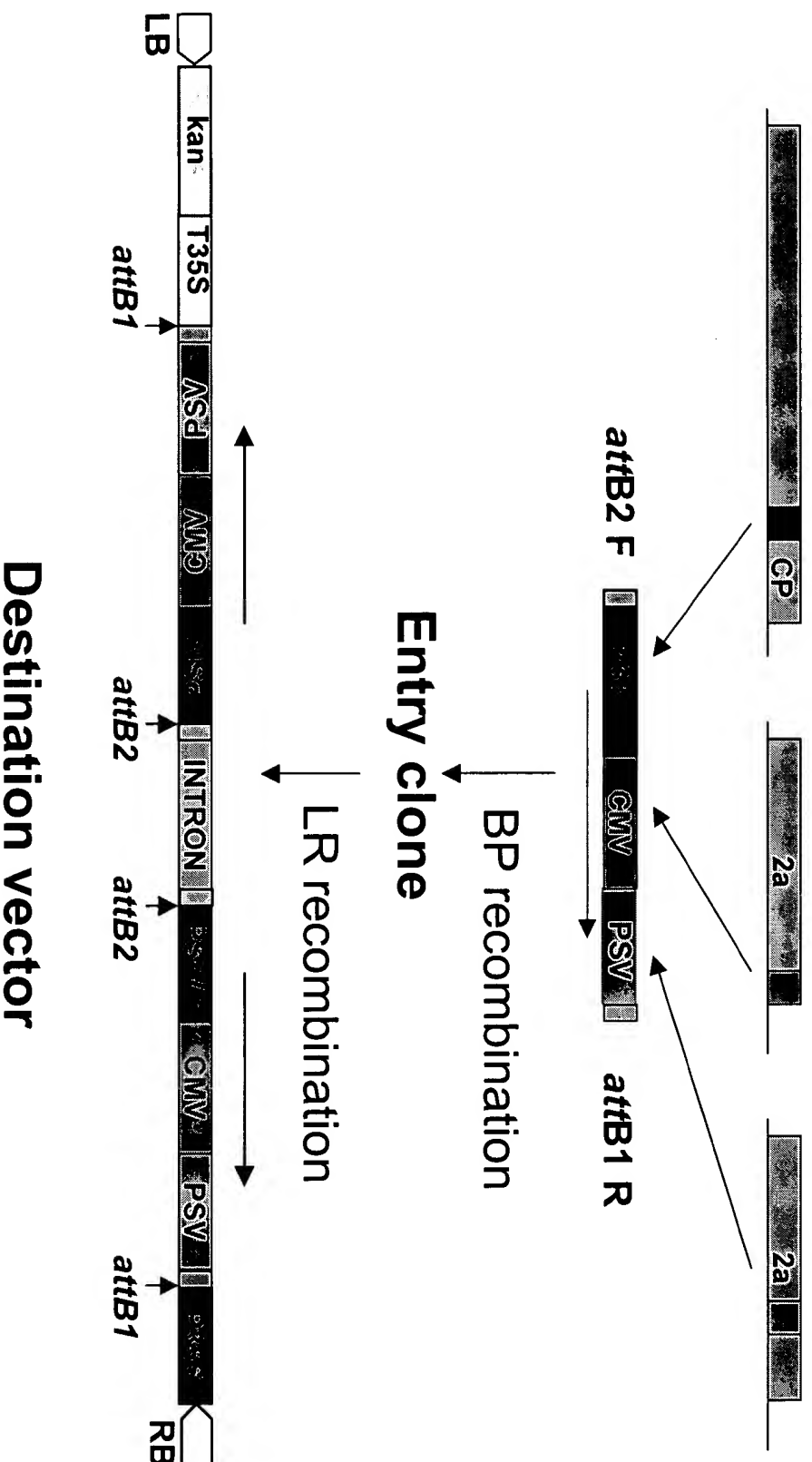


Resistance in difficult-to-transform plant feasible

Beware of silencing suppressors



Strategy to fuse different fragments of peanut viruses and transfer into inverted an IR binary vector using Gateway



Conclusions

TSMV and CMV can be stopped by enhanced gene silencing (transgenic resistance)

IR constructs are very powerful inducers of gene silencing

Stretches as short as 150 nt are sufficient for resistance

Linking viral sequences results in all-or-nothing resistance: resistance to one virus = resistance to all viruses

Very high resistance frequencies ($>80\%$ R_0) make easy transformation possible; even of 'difficult crops'

